THE HISTOLOGY AND ANATOMY OF THE REPRODUCTIVE SYSTEM OF THE LITTORAL GASTROPOD *BEMBICIUM NANUM* (LAMARCK) (FAM. LITTORINIDAE)

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Synopsis

In *Bembicium nanum* the sexes are separate and easily distinguishable ; the female by the yellow ovipositor, the male by the conspicuous penis.

The histology and anatomy of the male reproductive system of *B. nanum*, apart from the shape of the penis, the absence of penial glands, the compact nature of the testis and the germinal epithelium of the testis, are similar in general to other littorinids. However, in the female reproductive system, greater differences are found ; the renal oviduct is lined by a phagocytic syncytium, the receptaculum seminis is modified for storage, nourishment and phagocytosis of sperm, and the bursa copulatrix functions only as an organ for reception of spermatozoa.

Time of spawning appears to be independent of the time of year and different for each individual. Egg masses are slightly more numerous in the spring and summer months. Large seasonal differences in the size of the reproductive system and extensive resorption of gametes do not occur. In *B. nanum* resorption of spermatozoa is restricted to the vesicula seminalis of the male system and to the receptaculum seminis of the female system. Slight resorption of ova appears to occur in the renal oviduct of the female reproductive system of *B. nanum*. This limitation of phagocytosis of gametes in *B. nanum*, in comparison with North Sea littorinids, may be related to the milder climatic variations in the Sydney coastal areas.

INTRODUCTION

Little is known of reproduction and development in Australian littorinids (Anderson, 1960). Apart from a brief and rather inaccurate description by Kesteven (1902) and a brief reference by Anderson (1958) in a taxonomic survey of the genus *Bembicium*, no details of the reproductive system of *B. nanum* have yet been described. In the following work, the anatomy and histology of the reproductive system of *B. nanum* are described. Differences from North Sea littorinids are noted and related to continuous breeding throughout the year in *B. nanum*.

METHODS

Males and females of *B. nanum* were collected at intervals during 1961 and 1962 on the rock platforms of the ocean coast near Sydney. Animals, removed from their shells and relaxed in fresh water, were dissected under a binocular microscope. For histological studies, Smith's formol-bichromate, 5% formol saline and Baker's formaldehyde calcium were found to be the most suitable fixatives. To prevent hardening, material was taken to 95% alcohol, transferred to 1% celloidin in methyl benzoate, followed by benzene, then embedded in paraffin (M.P. 56°C). Sections were cut at 8μ and stained in Ehrlich's haematoxylin and eosin or Heidenhain's azan stain.

RESULTS

Male Reproductive System

As in all male prosobranchs, the testis (Fig. 1) in *B. nanum* lies in the visceral spire over the digestive gland, its tubules being grouped around the visceral arterial system (Anderson, 1958). The wall of each tubule is a flattened germinal epithelium. Within this lies a dense layer of spermatocytes, then a layer of spermatids, while mature spermatozoa (Fig. 2) and nurse cells (Fig. 3) with finely vacuolated cytoplasm and attached spermatids and spermatozoa occupy the lumen.

The small tubules unite and open into the coiled vesicula seminalis which runs along the axial surface of the spire and opens anteriorly into the vas deferens (Fig. 1).

The vesicula seminalis is about 200μ in diameter and is lined by cuboidal, vacuolated epithelial cells (Fig. 4). Spermatozoa are found in the vacuoles together with nurse cells, penetrating the epithelium, and in an unorientated mass in the lumen.

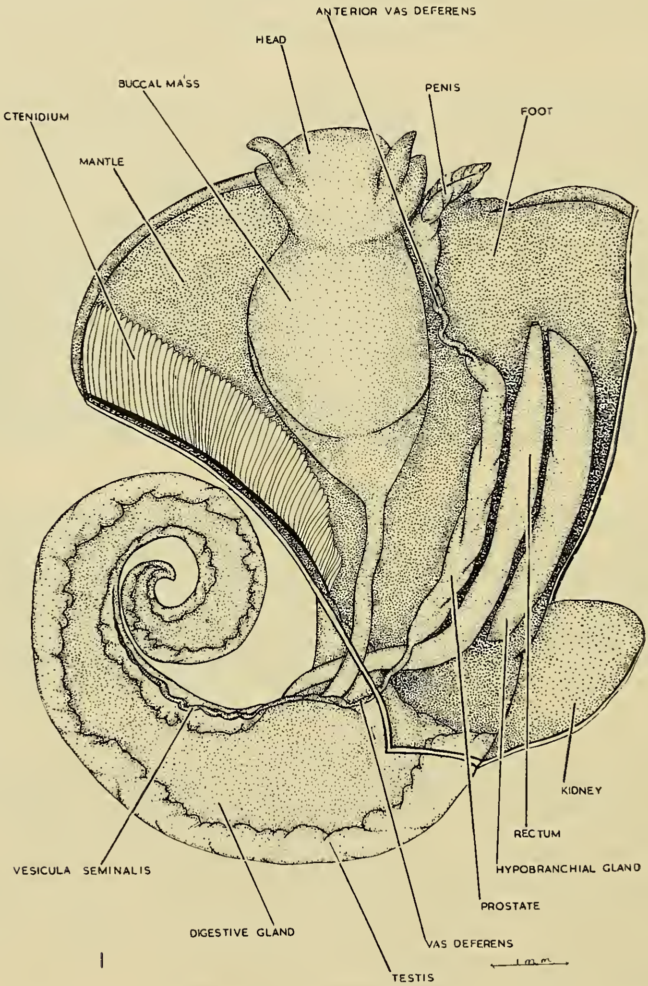


Fig. 1. Male reproductive system of *B. nanum*. The mantle cavity has been opened dorsally and the kidney has been folded to the right.

The vas deferens (Fig. 5) is short, about 100μ in diameter, lined by a ciliated cuboidal epithelium and surrounded by a thin layer of circular muscle, followed by a layer of connective tissue. It passes into the connective tissue under the kidney and continues as the prostate gland on the right side of the mantle cavity.

The prostrate gland looks like a complete duct in dissected specimens (Fig. 1), but is composed of an attached right and freely-hanging left lobe, separated ventrally so that the lumen of the gland opens into the mantle cavity. Each lobe has a deep ciliated sperm groove on its inner edge (Fig. 8).

Anteriorly, the prostate becomes a closed tube, the free edges of the two lobes fusing in the ventral midline.

The columnar epithelium lining the lobes of the prostate gland has two types of cells, gland cells and supporting cells (Fig. 6). Each gland cell has a basal nucleus, a single nucleolus and granular cytoplasm containing numerous eosinophil granules, especially in the narrower distal parts of the cell. The supporting cells, regularly placed between the gland cells, are expanded distally and compressed to thin cytoplasmic strands proximally. The free surfaces of the cells are densely ciliated, the cilia being longest on the edges of the sperm groove and in the groove itself. Many mucous cells are found in the epithelium

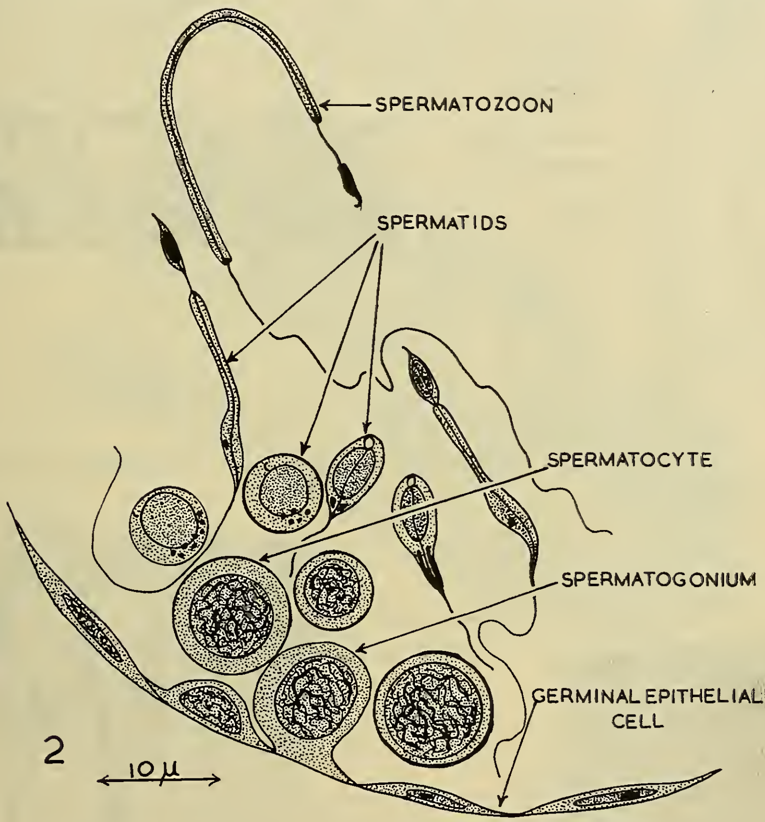
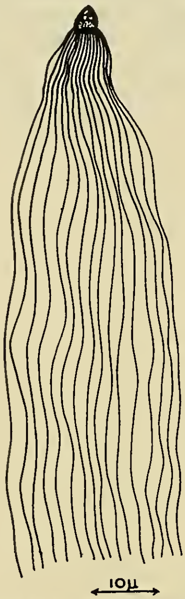


Fig. 2. *B. nanum.* T.S. through the wall of a testicular tubule.

of the edge of the left lobe (Fig. 7). No muscles appear under the prostate epithelium, but circular and longitudinal muscles are found under the mantle epithelium on the outer edge of the left lobe.

At the mouth of the mantle cavity, the prostate gland continues as the anterior vas deferens (Fig. 1), a narrow duct 75μ in diameter, lined by ciliated cuboidal epithelium and surrounded by a muscle sheath composed of circular, longitudinal and transverse muscle (Fig. 9). The anterior vas deferens passes along the foot to the right of the buccal mass, in a ridge of dense connective tissue and opens anteriorly into the penis.

The penis (Fig. 1), a conical projection slightly dorso-ventrally flattened, is covered by a ciliated columnar epithelium. Underlying the latter is a thin layer of circular muscle, then layers of dorso-ventral and oblique muscles, while longitudinal muscles run through the connective tissue internally. The connective tissue has numerous blood spaces and the penial nerve lies in a ventral position. The penial duct, which runs through the penis dorsally, is 200μ in diameter and is lined by ciliated columnar cells, 75μ high (Fig. 10). A thin coat of circular, longitudinal and transverse muscles lies immediately external to this epithelium.



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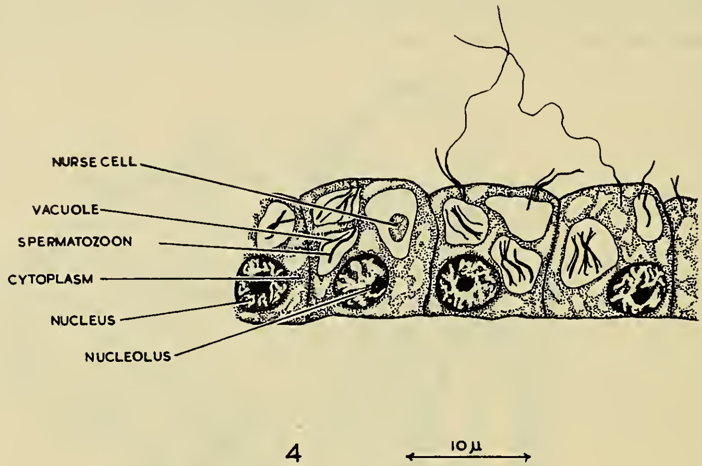

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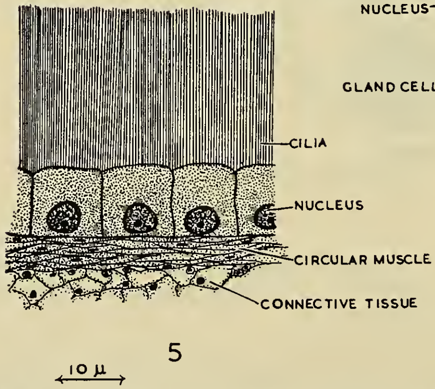
Figs 3, 4. *B. nanum.* 3, Nurse cell with attached spermatozoa ; 4, T.S. through the wall of the vesicula seminalis.



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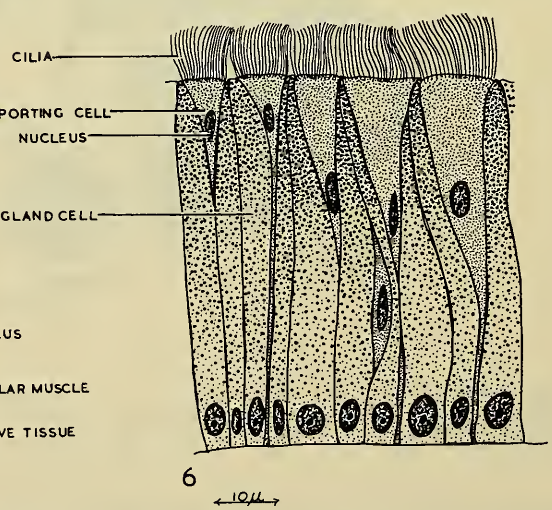

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Figs 5, 6. *B. nanum.* 5, T.S. through the wall of the vas deferens ; 6, T.S. through the wall of the prostate gland.

Female Reproductive System

Like the testis, the ovary (Fig. 11) lies over the digestive gland and its tubules are grouped around the visceral arterial system, this arrangement being seen in young specimens only (Anderson, 1958). The wall of each tubule is a flattened germinal epithelium, with developing oocytes projecting into the lumen of the tubule and mature oocytes lying in the lumen (Fig. 12). The ovarian tubules open into a single duct, the renal oviduct (Fig. 11), which runs through the connective tissue under the kidney.

The wall of the renal oviduct is composed of a syncytial epithelium with scattered oval nuclei, each with a single nucleolus, and an external sheath of circular muscle. Large vacuoles, 10μ in diameter, filled with large, yolky, eosinophil granules, also occur in the epithelium cytoplasm (Fig. 15). Anteriorly, the renal oviduct dilates, then narrows and opens into the pallial oviduct or "uterus" (Fig. 11). In its narrower region the renal oviduct has a typical ciliated cuboidal epithelium.

Immediately behind the opening of the renal oviduct into the pallial oviduct is the opening of the receptaculum seminis (Fig. 11). This is a short blind duct lying in the mantle cavity between the renal oviduct and pallial oviduct, in which it is embedded anteriorly. Posteriorly, the receptaculum

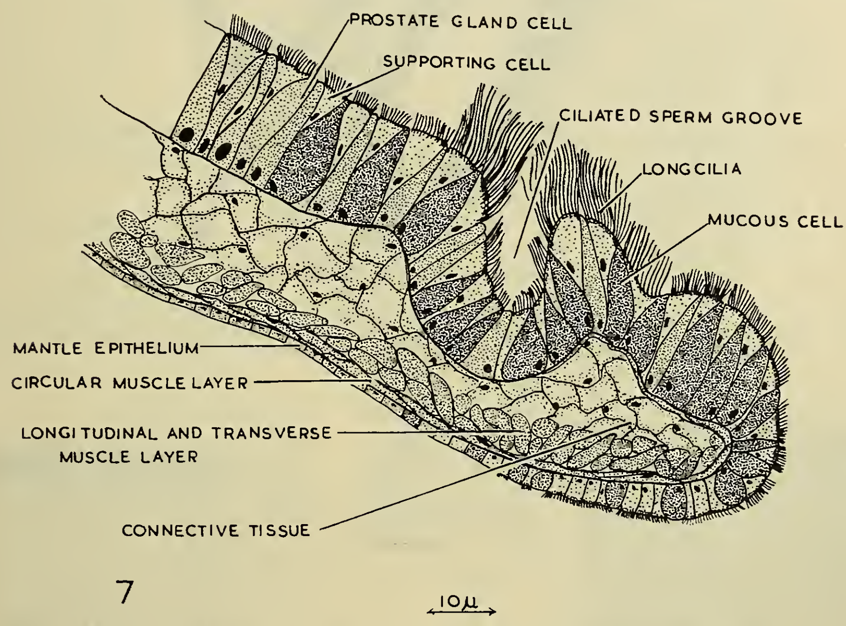


Fig. 7. *B. nanum.* T.S. through the left lobe of the prostate gland.

seminis ends in a bulb, 300 μ in diameter, lined by unciliated, cuboidal epithelial cells and filled with unorientated spermatozoa (Fig. 17). Anteriorly, it is laterally compressed, with diameters of about 300μ and 150μ (Fig. 13). In this anterior portion, its ventral epithelium, which lines a deep groove, is a syncytium with large oval nuclei. Numerous spermatozoa are found with their heads embedded in the syncytium (Fig. 16). Dorsally, the epithelium is columnar and is composed of three cell types : mucous cells, cells containing granules and spermatozoa, and ciliated supporting cells (Fig. 14). The mucous cells each have a granular cytoplasm staining evenly with haematoxylin and a basal nucleus containing a single nucleolus. The cells containing granules and spermatozoa, presumably phagocytically digesting the latter, also have a basal nucleus containing a single nucleolus, but their cytoplasmic granules stain strongly with eosin. Neither of these cell types is ciliated. The supporting cells, between them, are expanded distally and bear cilia about 10μ high. A circular muscle sheath, 10μ thick, surrounds the receptaculum seminis.

The pallial oviduct has, running ventrally along its length, a channel with densely ciliated cuboidal epithelium, 5μ high (Fig. 18). Its lateral and dorsal walls, on the other hand, are enlarged to form a posterior albumen gland and an anterior jelly gland. The two glands lie to the right of the rectum on the dorso-lateral wall of the mantle cavity and can be seen through the thin mantle tissue (Fig. 11). The albumen gland (Fig. 19) is a large mass of much folded epithelium, the lumen between the epithelial folds connecting with the ventral

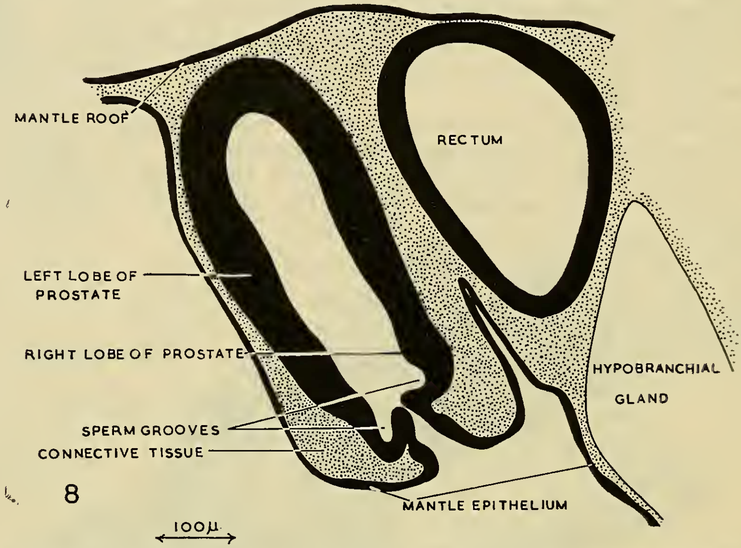
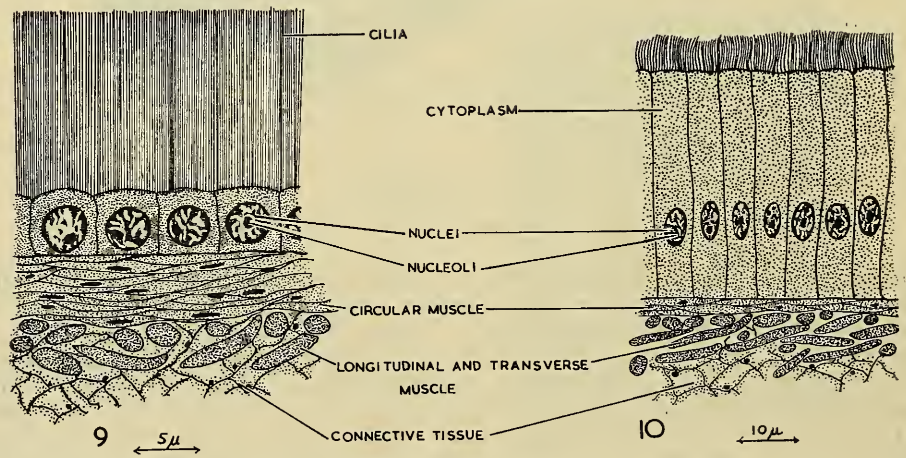


Fig. 8. *B. nanum.* Diagrammatic T.S. through the roof of the mantle cavity showing the relative positions of the prostate gland, rectum and hypobranchial gland.



Figs 9, 10. *B. nanum.* 9, T.S. through the wall of the anterior vas deferens ; 10, T.S. through the wall of the penial sperm duct.

channel of the oviduct. Both glandular and supporting cells occur in the epithelium. The densely ciliated supporting cells are each expanded distally and are connected to the basement membrane by thin cytoplasmic connections and have a spindle-shaped, densely staining nucleus at the base of the distal expansion. The albumen gland cells are filled with secretion droplets staining heavily with haematoxylin, these droplets being more numerous in the distal parts of the cells. The nucleus of each cell is oval, basal and has a single nucleolus. The epithelium of the albumen gland is covered externally by a coat of circular muscle, 5μ thick.

The jelly gland (Fig. 11) is composed of the two thickened, folded, lateral walls of the pallial oviduct, with the lumen between them opening into the ventral channel of the pallial oviduct. The gland cells (Fig. 20) are filled with

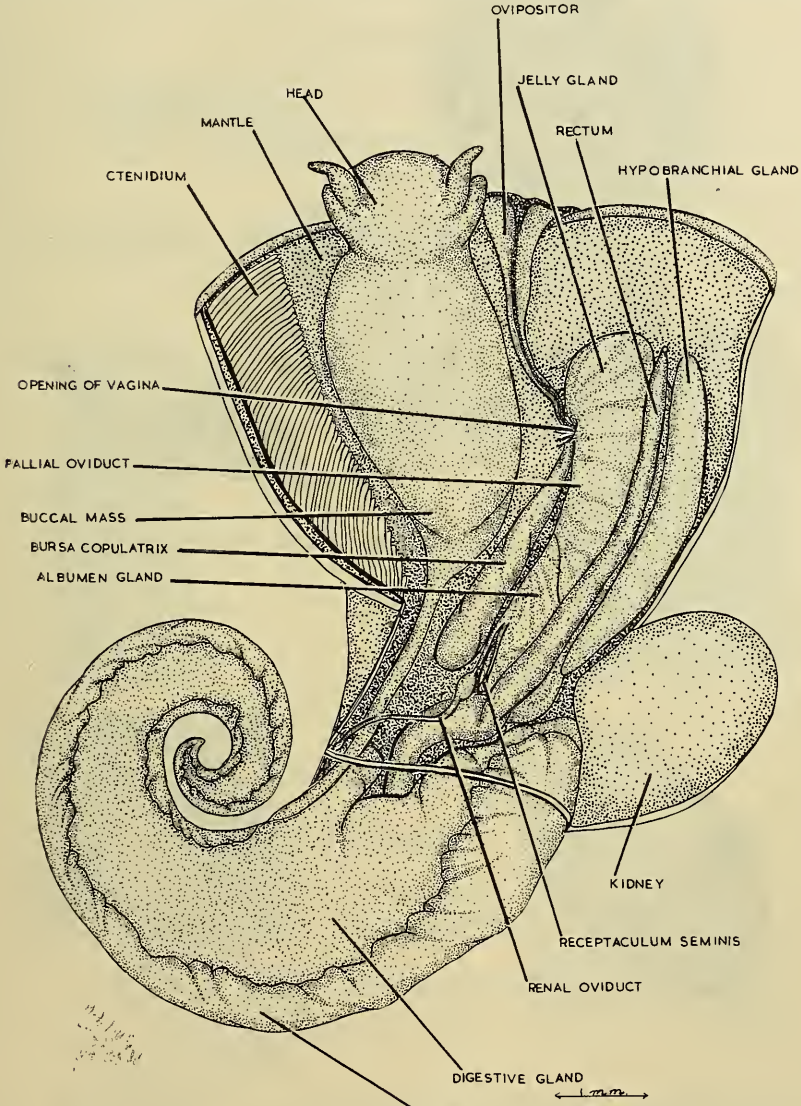


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Fig. 11. Female reproductive system of *B. nanum*. The mantle cavity has been opened dorsally and the kidney has been folded to the right.

secretion droplets which stain heavily with haematoxylin. The supporting cells are similar to those of the albumen gland.

Opening anteriorly into the ventral channel of the pallial oviduct is the bursa copulatrix, a large blind duct, 300μ in diameter, which lies to the right of the oviduct in the mantle cavity (Fig. 11). Its ciliated columnar epithelium (Fig. 22) is thrown into folds of varying size (Fig. 21), which are supported by

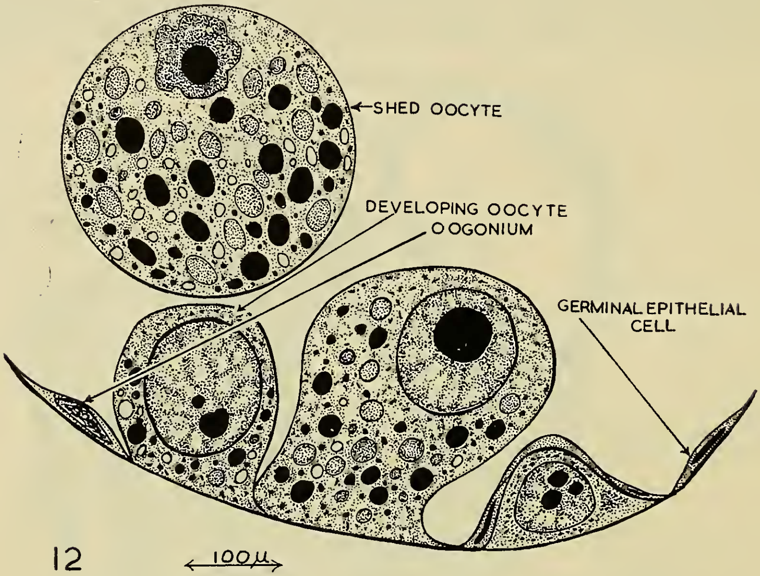
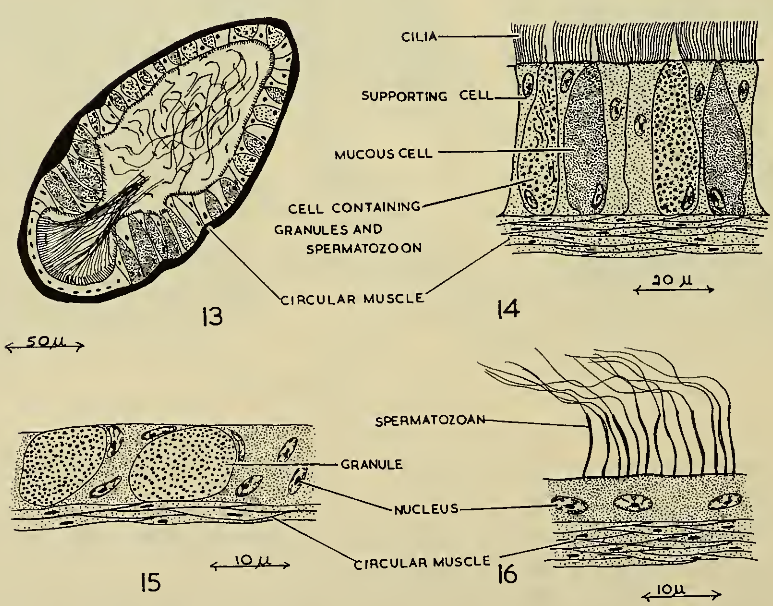


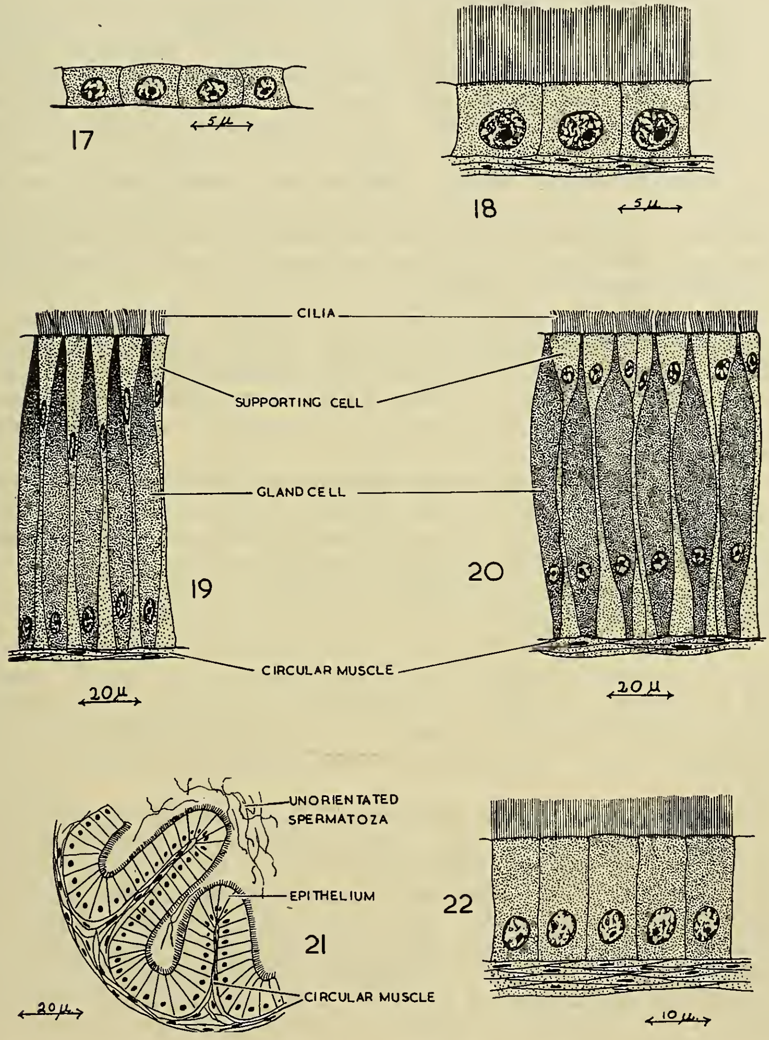
Fig. 12. T.S. through the wall of an ovarian tubule of *B. nanum*.



Figs 13–16. *B. nanum*. 13, T.S. through the receptaculum seminis ; 14, T.S. through the dorsal epithelium of the receptaculum seminis ; 15, T.S. through the wall of the renal oviduct ; 16, T.S. through the ventral syncytium of the receptaculum seminis.

extensions of muscle from a surrounding muscular sheath. Unorientated spermatozoa are found in the lumen of the bursa copulatrix.

At the mouth of the mantle cavity the bursa and oviduct unite as a short vagina (Fig. 11). From this, a ciliated groove runs between two lobes on the foot, to the right of the buccal mass and head. These lobes comprise the



Figs 17–22. *B. nanum.* 17, T.S. through the wall of the posterior bulb of the receptaculum seminis ; 18, T.S. through the wall of the ventral channel of the pallial oviduct ; 19, T.S. through the wall of the albumen gland ; 20, T.S. through the wall of the jelly gland ; 21, diagrammatic T.S. through the wall of the bursa copulatrix ; 22, T.S. through the wall of the bursa copulatrix.

ovipositor. Each lobe has a ciliated epithelium, 50μ high, in which many mucous cells are found. The epithelium at the edge of the groove is similar, but about 20μ high. Beneath the epithelium of the ovipositor is a ridge of circular and transverse muscle.

Numerous large, granule-filled cells were recorded in the tissues of the reproductive system of *Littorina rudis* by Linke (1933), who supposed them to be amoebocytes of excretory function. Similar cells were seen in the tissues of *B. nanum*, especially in the epithelium of the albumen gland, jelly gland, ventral channel of the pallial oviduct and bursa copulatrix.

Discussion

Male Reproductive System

The histology and anatomy of the reproductive system of *B. nanum* are similar in general to those of *Littorina littorea*, *L. obtusa*, *L. rudis* and *Cremnoconchus syhadrensis*, described by Linke (1933, 1935). Anderson (1958), however, has already noted the following differences between the male reproductive systems in *Littorina* and *Bembicium* : the shape of the penis, the absence of penial glands and the compact nature of the testis, which lies over the digestive gland in *Bembicium.* Anderson's results are confirmed here and further differences have also been noted. The germinal epithelium in the testis of *B. nanum* is a flattened epithelium, not the syncytium described by Linke in other littorinids. Linke also described seasonal phagocytosis of spermatozoa in the testes of littorinids. No seasonal phagocytosis of spermatozoa was observed in the testis of *B. nanum.*

As in *L. littorea*, *L. obtusa*, and *L. rudis* (Linke, 1933) and in *Ocinebra erinacea*, *Nucella lapillus*, *Nassarius reticulatus* and *Buccinium undatum* (Fretter, 1941), phagocytosis of spermatozoa and degenerating nurse cells occurs in the vesicula seminalis of *B. nanum.* The muscular vas deferens apparently acts as a sphincter, regulating flow of spermatozoa from the vesicula seminalis into the prostate.

Kesteven (1902), who briefly described the male reproductive system of *B. nanum*, made no reference to the prostate, referring to it as a closed vas deferens. In fact, the prostate is open and glandular. Spermatozoa move along the ciliated groove of the prostate gland and receive the prostate secretion before moving into the closed penial sperm duct.

Female Reproductive System

Kesteven's (1902) description of the anatomy of the female reproductive system of *B. nanum* contained a number of errors, namely, his attribution of reduction in overall size of the system to non-breeding periods, his description of the " uterus " and his omission of the receptaculum seminis and bursa copulatrix.

As in *L. obtusa* and *L. rudis* (Pelseneer, 1911 ; Linke, 1933), sexual ripeness is independent of the time of year and is different for each individual in *B. nanum.* Absence of seasonal phagocytosis of eggs in the ovary of *B. nanum* (cf. other littorinids, Linke, 1933) may possibly be due to the milder climatic variations in the Sydney coastal area, as compared with those in the North Sea coastal areas. Kesteven (1902) noted a marked overall reduction in the size of the female reproductive system, especially in the ovary, but no such reduction was noted in this examination of *B. nanum.* His diagram of the female system appears to be of an immature specimen and his observations on size reduction were probably due to either immaturity or examinarion of parasitized specimens.

No phagocytosis of eggs occurs in the renal oviduct of other littorinids (Linke, 1933), but Fretter (1941) described it in the ingesting gland of other prosobranchs. While no ingesting gland is found in *B. nanum*, phagocytosis of eggs appears to occur in the renal oviduct.

Using the methods outlined above, the difficulties encountered by Linke in the histological examination of the " uterine " glands in the pallial oviduct were overcome. Using these techniques, it was possible to identify the anterior jelly gland and the posterior albumen gland in the pallial oviduct of *B. nanum.* A similar arrangement of glands was found in the pallial oviduct of *B. auratum* by Anderson (1958), but she gave no details of histological structure. The enlarged " uterus " recorded by Kesteven in *B. nanum* is in fact these glands of the pallial oviduct. The muscular supporting bands he described are not muscular tissue, but folds of glandular material (Fig. 11). The glands are surrounded by a very thin coat of muscular tissue. During secretion of the albumen gland in *Littorina*, Linke described extrusion of the upper parts of the gland cells into the lumen of the gland. Presumably, this was a fixation artifact as no such extrusions are found in the albumen gland of *B. nanum*. The structure of the jelly gland is similar to that described by Linke (1933) in *L. obtusa* and *L. littorina*.

Kesteven (1902) made no reference to the receptaculum seminis and bursa copulatrix in *B. nanum*. The position of the receptaculum seminis and bursa copulatrix is similar to that recorded by Anderson (1958) in *B. auratum*. In *Littorina* Linke (1933) described a receptaculum seminis lined by a syncytial epithelium, in which sperms are embedded. The receptaculum of *B. nanum* differs from that of *Littorina* in a number of ways : in *B. nanum* the receptaculum swells to form a bulb lined by cuboidal epithelium and filled with unorientated spermatozoa ; the anterior part is lined ventrally by a syncytium in which spermatozoa are embedded, and dorsally by an epithelium composed of mucous and ciliated supporting cells, and cells containing granules and spermatozoa. The latter cells are presumably phagocytic. No similar receptaculum seminis has been described in other prosobranchs.

Unlike the bursa copulatrix of *Littorina*, which is lined by a syncytium and contains orientated spermatozoa (Linke, 1933), the bursa copulatrix of *B. nanum* is lined by a ciliated columnar epithelium, contains unorientated spermatozoa, and functions only as a receptor organ for spermatozoa.

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References

Anderson, D. T., 1960.—The life histories of marine prosobranch molluscs. *Journ. Malacol. Soc. Aust.*, 4 : 16–29.

Anderson, H., 1958.—The gastropod genus *Bembicium* Phillipi. *Aust. J. Mar. Freshw. Res.*, 9 : 546–568.

Fretter, V., 1941.—The genital ducts of some British stenoglossen prosobranchs. *J. Mar. Biol. Assoc. U.K.*, 25 : 173–211.

Kesteven, H. L., 1902.—Notes on Prosobranchiata, No. 2 Littorinacea. Proc. Linn. Soc. N.S.W., 27 : 620–636.

Linke, O., 1933.—Morphologie und Physiologie des Genitalapparates der Nordseelittorinin. *Wiss. Meeresuntersuch.*, 19 : 1–60.

Linke, O., 1935.—Zur Morphologie und Physiologie des Genitalapparatus der Susswasser-littorinide *Cremnoconchus syhadrensis* Blanchford. *Arch. Naturgesch.*, 4 : 72–87.

Pelseneer, P., 1911.—Reserches sur l'embryologie des Gastropodes. *Bruxelles Mem. Acad. Roy.*, Ser. 2, 6 : 1–167.